



REPORT

No. T 254/42  
Code 31-06-42

Acute Oral Toxicity of Puerine

Submitted to

Natural Product.

Thailand Institute of Scientific and Technological Research  
(TISTR)

August 1999



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## REPORT

Title : Acute oral toxicity study of Puerine  
Product Identification : Tonic drug  
Product Description : Dried plant odor and yellow brown powder  
Report and Study No. : T 254/42, Code 31-06-42  
Client : Natural Product  
Date of Contact : June 23, 1999  
Date of Test : July 14-29, 1999  
Protocol No. : 6/1999  
Method : Acute oral toxicity test, Limit test, OECD, 1993

### Summary :

The test material, Puerine (MFG lot #PU-002), was received on June 23, 1999.

The acute oral toxicity test was conducted in young adult Sprague-Dawley rats on both sexes. All rats were dosed at 2,000 mg/kg-body weight according to OECD guidelines, 1993. The rats were observed at 1/2, 1 and 3 hours after dosing and once daily for 14 days.

No toxic signs and no mortalities occurred during 14 days of observation period.

No gross pathological changes were observed at necropsy.

The above results are valid exclusively for tested/analysed samples as mentioned in this report  
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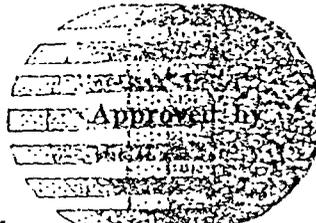
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## ACUTE ORAL TOXICITY STUDY OF PUERIN

### 1. Materials

#### 1.1 Test material

The test material, Puerine, tonic drug (MFG lot #PU-002), was received on June 23, 1999. It was dried plant odor and yellow brown powder. The test material was tested as suspension in distilled water, 10 % (w/v).

#### 1.2 Animals

Healthy Sprague Dawley rats were purchased from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakhonpathom. Initial body weight ranges were 300-350 grams for male and 200-230 grams for female (one day before fasting).

#### 1.3 Food, Pokphand Animal Feed Co., Ltd., Thailand

#### 1.4 Drinking water, filtered water

#### 1.5 Distilled water

#### 1.6 Balance, Mettler AE 160, CH 8006, Mettler Instruments AG, Switzerland

#### 1.7 Surgical instruments

#### 1.8 10 ml disposable syringe, Nipro Shoji Kaisha Ltd., Japan

#### 1.9 Stomach tubes, Ogawa Seiki Co Ltd., Japan

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## 2. Methods

The experiment was conducted in accordance with the acute oral toxicity test, limit test, OECD guidelines, 1993.

Ten rats in each group, five rats in each sex, were acclimatized to the laboratory environment for one week at room temperature of  $24 \pm 1$  °C. A random number was allocated to each rat on receipt. Each rat was uniquely identified by tail tattoo. The rats were fasted for 16 hours prior to treatment. Drinking water was available *ad libitum*.

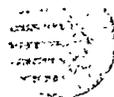
The test material was administered orally at the dose of 2,000 mg/kg body weight using a calibrated syringe and a stomach tube. The dosage of the test material was calculated based on the fasted body weight of rats. Control rats were dosed with distilled water at equivolume as experimental rats.

After dosing, each rat was returned to its designated cage. Feed was replaced approximately 4 hours thereafter. Feed and water were provided *ad libitum* for the balance of the study. The rats were observed 24 hours after dosing and once daily for 14 days thereafter for signs of gross toxicity and mortality. Body weights of rats were recorded on day 1 (prior to dosing), day 8 and day 15 (at termination) or after death.

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Gross necropsies were performed on all decedents and all survivors at terminal sacrifice. All survivors to termination were euthanized by CO<sub>2</sub> inhalation.

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### 3. Results

After dosing, all rats appeared normal and survived to termination.

The summary of mortality data are shown in Table 1. The body weights on day 1 (initial), day 8 and day 15 (termination) are shown in Table 2. Necropsy findings on the survivors at terminal sacrifice are shown in Table 3.

Table 1 Summary of mortality data

Test Materials	Mortality		
	Male	Female	Total
Distilled water	0/5	0/5	0/10
Puerine (2,000 mg/kg body weight)	0/5	0/5	0/10

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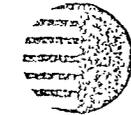
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Table 2 Body weight of rats at initial and termination of the study

Test Materials	Animal No.	Sex	Body weight (g)		
			Day 1*	Day 8	Day 15
Distilled water	1	Male	290	332	364
	2	Male	302	358	397
	3	Male	288	342	384
	4	Male	310	350	386
	5	Male	310	372	422
	6	Female	200	238	254
	7	Female	214	244	250
	8	Female	210	236	246
	9	Female	192	224	238
	10	Female	210	230	248
Puerine (2,000 mg/kg body weight)	11	Male	284	330	364
	12	Male	302	352	382
	13	Male	298	360	388
	14	Male	322	382	412
	15	Male	320	378	416
	16	Female	204	230	254
	17	Female	190	210	222
	18	Female	210	240	260
	19	Female	208	228	251
	20	Female	192	220	235

\*fasted body weights

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Table 3 Necropsy observation

Test material	Animal No.	Sex	Gross pathological findings
Distilled water	1	Male	Normal
	2	Male	Normal
	3	Male	Normal
	4	Male	Normal
	5	Male	Normal
	6	Female	Normal
	7	Female	Normal
	8	Female	Normal
	9	Female	caecum : slight gaseous distension
	10	Female	Normal
Puerine (2,000 mg/kg body weight)	11	Male	Normal
	12	Male	Normal
	13	Male	Normal
	14	Male	Normal
	15	Male	Normal
	16	Female	Normal
	17	Female	Normal
	18	Female	Normal
	19	Female	Normal
	20	Female	Normal

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#### 4. Discussion

It was found that all rats treated with Puerine appeared normal.

OECD (1993) claimed that in the case of compound-related mortality is produced, a full study may need to be considered. In addition, Auletta (1995) stated that limit dose, according to EPA and OECD, is considered high enough that if no mortality or significant toxicity is seen in animals receiving this dose, no higher doses are required.

#### 5. Summary

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No toxic signs and ~~no mortality~~ were observed during 14 days of observation period.

No gross pathological changes were observed at necropsy.

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## 6. References

1. Organization for Economic Co-operation and Development. 1993. OECD Guidelines for testing of chemicals, Volume 2, Section 4, Health Effects, 401. Acute Oral Toxicity.
2. Auletta, C.S. 1995. Acute, Subacute and Chronic Toxicity. In: CRC Handbook of Michael J. Derelanko and Manfred A Hollinger, eds., CRC Press, pp. 51-104.

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